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The Bacterial Degradation of Nicotine and Related Compounds

The following informal summary of our investigations conducted since the preparation of the annual report for 1958-1959 is submitted as our semi-annual report. During this period we have completed our work on the third oxidative product, shown the involvement of a K- or Q-like vitamin in two of the oxidative steps studied, and obtained more information on the fourth oxidative step. Our third publication in the nicotine series is now in print (J. Biol. Chem., 235:795-80, 1960) and reprints will be sent as soon as they arrive. A report of the work on the third product will be presented at the national meeting of the Society of American Bacteriologist, May 3, in Philadelphia, and an abstract of this paper was published in Bacteriological Proceedings, p. 168 (1960).

A. The third oxidative product.

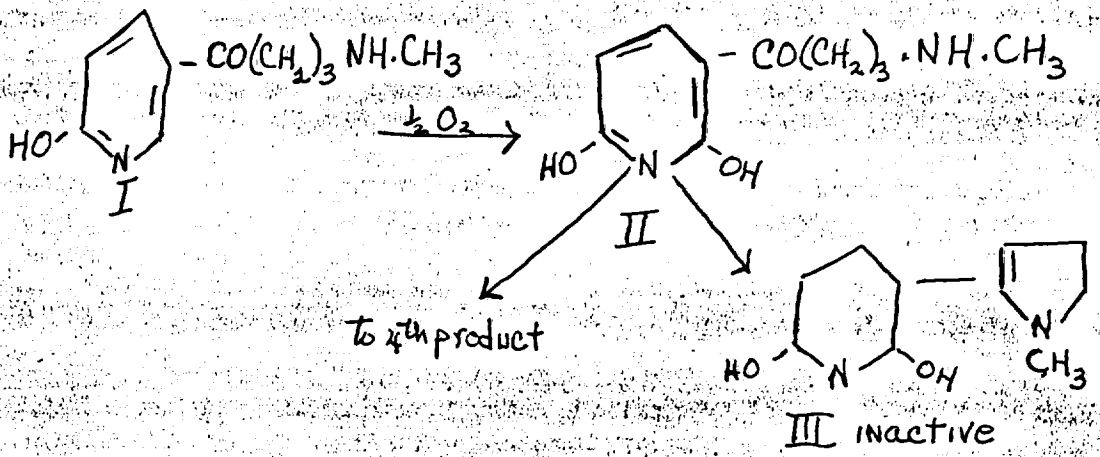
The previous annual report described the isolation in crystalline form of a compound then believed to be the third oxidative product. This compound was tentatively identified as 2,6-dihydroxy-n-methylmyosmine. Further proof of its identity has been obtained by selective reduction of the double bond in the pyrrolidine portion of the molecule by means of $NaBH_4$, to form a 3-substituted dipyridol. Since the substituent on the 3 position of the pyridine ring is saturated, it would not be expected to contribute significantly to the ultraviolet absorbtion spectrum of the reduced product, which turned out to have identical absorbtion characteristics to that of 2,6-dipyridol. This evidence makes conclusive the former tentative identification of the isolated product.

As mentioned in the previous report, the above mentioned compound was not further oxidized by our bacterium or by extracts thereof. It has now been established that this compound is actually a side product of the true third intermediate, formed by a nonenzymatic, nonoxidative, alteration of the true intermediate. When conversion of the second to third product is carried out in a spectrophotometer measuring increase in absorbance at 360 mu (the maximum of 2,6-dihydroxy-n-methylmyosmine), two distinct rates separated by a sharp break are observed. The initial rate is very rapid and the second rate quite slow. A total spectrum run on reaction mixtures at the break point revealed a new substance present with the following properties:

1. Ultraviolet absorbtion maxima at 345 and 275 mu
2. A carbonyl function is present
3. The compound is irreversibly converted nonenzymatically and nonoxidatively to the previously isolated 2,6-dihydroxy-n-methylmyosmine under the conditions employed for its enzymatic formation.
4. This irreversible conversion occurs instantaneously with the application of heat or alkalie.
5. Addition of acid to this material results in the formation of a compound (probably a salt) with a single absorbtion maximum at 320 mu.

6. Addition of a 0-40 ammonium sulfate fraction of the crude extract results in the rapid disappearance of its absorbancy at 345 mu.

We have as yet been unable to isolate this unstable compound which we conclude is the active third intermediate in our system. Based on the presence of a carbonyl function in the active molecule and its nonoxidative conversion to the identified dihydroxymyosmine, we propose the following sequence for the formation and transformation of the active third intermediate, 2,6-dihydroxypseudooxynicotine:



I. 6-hydroxypseudooxynicotine (2nd product)
II. 2,6-dihydroxypseudooxynicotine (3rd product)
III. 2,6-dihydroxy-n-methylmyosmine

B. Involvement of a K- or Q-vitamin.

It has been mentioned in previous reports that extracts obtained from our bacterium fail to catalize the first or third oxidative steps in nicotine breakdown unless they are supplemented with a redox dye such as methylene blue or brilliant cresyl blue. The nature of the cofactor being substituted for by the dye remained unknown despite numerous attempts to uncover its identity. It was recently found that several vitamin K analogues including menadione and juglone will replace the redox dyes formerly used as accesory hydrogen acceptors in these reactions. These coenzymes catalize the reactions at rates considerably higher than those obtained using redox dyes.

Various extracts of freshly grown cells made with lipid solvents have been found to be active in this respect also. The ultraviolet spectra of these cell extracts correspond to the general pattern exhibited by coenzyme Q type compounds suggesting that such a compound is the actual cofactor. The fact that synthetic vitamin K is active does not contradict the above statement since the Q and K molecules are very similar in structure and function. If substantiated, these data establish a new function for such vitamins as being involved in hydroxylation reactions.

C. The 4th intermediate.

Recent spectrophotometric data indicate that a new compound is formed when the 345 mu material (II), formed in a cuvette, is subjected to the action of enzymes present in a 0-40 ammonium sulfate fraction. A new ultraviolet maximum at 325 mu appears and in turn disappears, suggesting the temporary accumulation and further conversion of the next intermediate. The data, which is not yet conclusive, suggests a hydrolytic opening of the substituted pyridine ring to yield the 325 mu material followed by an oxidation that destroys all ultraviolet absorbtion peaks.

D. Projected work.

In the next six months we hope to carry out the following investigations:

1. Further studies on blue pigment formation on which no work has been done since last summer, with special emphasis on the isolation of the pigment in pure form.

2. Isolation of the third intermediate, probably as a phenylhydrazone.

3. Attempts to isolate and identify the "Q-like" coenzyme.

4. Investigation of the fourth and fifth oxidative reactions following the pattern of work with the previous three steps.